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13. ABSTRACT *(Maximum 200 words)*

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Design, Materials, and Methods: **Silver–nylon wound dressings were used as either anodes (−) or cathodes (+) on 20% total body surface area full-thickness scalds in anesthetized male Sprague-Dawley rats. Untreated burned rats and rats treated with silver–nylon dressings without current were used as controls.**

Measurements and Main Results: **Immediately applied, continuous DC reduced burn edema by 17 to 48% at different times up to 48 hours postburn ($p < 0.001$). Neither reversal of electrode polarity nor change in current density had any significant effect on the results of treatment. Starting treatment during the first 8 hours postburn produced the least edema accumulation, but the reduction was significant even when DC was applied 36 hours afterburn. If started immediately after injury, treatment had to be continued a minimum of 8 hours to be most effective.**

Conclusions: **Direct electric current has a beneficial effect in reducing wound edema after burn injury.**

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Direct Current Reduces Wound Edema after Full-Thickness Burn Injury in Rats

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Conclusions: **Direct electric current has a beneficial effect in reducing wound edema after burn injury.**

Because of the complex and varied factors involved, the pathophysiology of postburn edema formation is not completely understood. However, there are many reports that reduction of burn wound edema can be accomplished using physical, biochemical, humoral, and cellular mediators (cold,[1,2] catalase, superoxide dismutase,[3] free radical scavengers,[4] platelet activating factor antagonist,[5] ganglionic blocking agents,[6] connective tissue hydrolytic enzymes, hyaluronidase,[7] or vitamin C[8]). In the past few years, we have reported antimicrobial and wound healing effects of direct current (DC).[9–11] This paper presents data on the changes in wound edema attending application of DC of different intensity and polarity to full-thickness scald burns.

Previous experiments have shown that after 48 hours postburn (PB), desiccation, coagulation, and inflammation developing in full-thickness burns influence edema volume, therefore, observations during the first 48 hours were emphasized.

MATERIALS AND METHODS

Animal Model

A total of 1,860 male Sprague-Dawley rats of 225 g average weight were used. Rats were anesthetized with sodium pentobarbital (38 mg/100 g of body weight) administered intraperitoneally, then were clipped and depilated. Full-thickness dorsal scald wounds of 20% of the total body surface area (TBSA) were produced by exposure to boiling water (100°C) for 10 seconds using a Walker-Mason template.[12] All animals were kept in individual cages in the animal intensive care unit (temperature 78°–80°F, relative humidity ~50%) and were allowed food and water ad libitum. No resuscitation was given. All animals recovered uneventfully.

Direct Current Exposure

The wounds were covered with silver–nylon cloths soaked in physiologic saline (wet silver–nylon (SN)) that served as electrodes (anode (−) or cathode (+)). The counter electrode (silver–nylon) was placed on the shaved abdomen. Isolated wires were clipped to the center of the dressings and connected to a previously described direct current source[13] supplying eight sets of circuit connections. Constant direct current (−4, −40, or +40 μA) was applied to the burn wounds continuously. Voltage fluctuation was less than 1 Volt. Limitation of animal activity inside the cages was minimal during treatment.

Experimental Groups

Three sets of experiments were performed. In the first set, continuous direct current was started immediately postburn (PB). Edema was measured at 15 minutes, 30 minutes 1, 2, 3, 4, 6, 8, 12, 24, 36, or 48 hours PB in all control and DC treated groups as listed in Table 1.

The second set, as shown in Table 2, included two series of experiments. In the first series, SN was applied immediately PB and DC was started at 30 minutes, 1, 2, 3, 4, 6, 8, 12, 24, or 36 hours PB and edema was measured at 48 hours. In the second series, SN was applied immediately PB and continuous DC was started at 12 hours PB and maintained during the remaining times before edema measurements. Edema was measured 15 minutes, 1, 2, 4, 6, 8, 12, 24, or 36 hours after

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In conducting the research described in this report, the investigators adhered to the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and with the *Guide for the Care and Use of Laboratory Animals*, National Institutes of Health Publication 85-23.

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TABLE 1. **Experimental groups for edema study of full-thickness burns in rats when direct current applied immediately postburn[a]**

Group	Description	No. of Rats
Controls		
Nonburn control	Nonburned skin	30
Burn control	Burn only	390
Wet silver–nylon control (SN)	Burn + SN + saline	390
Treatments		
−40 μA DC	Burn + −40 μA DC	140
−4 μA DC	Burn + −4 μA DC	130
+40 μA DC	Burn + +40 μA DC	130

[a] First set of the experiment.
DC = direct current; SN = silver–nylon.

DC application. In the control (wet SN) group, edema was measured 11 times from 30 minutes to 48 hours PB.

In the third set, DC applied immediately PB was disconnected at 1, 2, 4, 6, 8, 10, 12, 24, or 36 hours PB (with SN remaining on the wound), and edema measurements were done at 48 hours PB. Edema measured at 48 hours PB without DC applied was used as control. Experimental groups are listed in Table 3.

Edema Measurements

At intervals of up to 120 hours after injury, the rats were reanesthetized, and the entire burn wound, including the panniculus carnosus, was excised, immediately was weighed (wet weight, W), and then was dried to constant weight (dry weight, D) in an electric oven for 4 days at 70°C. After skin samples were taken, the rats were given an overdose of an intravenous injection of sodium pentobarbital (40 mg). Ten animals were utilized for edema analysis at each examined time of each experimental control or DC treated group.

TABLE 2. **Experimental groups for edema study of full-thickness burns in rats when direct current application delayed[a]**

Group	Description	No. of Rats
Controls		
Wet silver–nylon control	Burn + SN + saline	110
Treatments		
Series 1		
−40 μA DC	Burn + −40 μA DC	110
−4 μA DC	Burn + −4 μA DC	110
+40 μA DC	Burn + +40 μA DC	110
Series 2		
−40 μA DC	Burn + −40 μA DC begun at 12 hours PB	80

[a] Second set of the experiment.
DC = direct current; SN = silver–nylon.

TABLE 3. **Experimental groups for edema study of full-thickness burns in rats at 48 hours afterburn when direct current application disconnected at various times[a]**

Group	Description	No. of Rats
Controls		
Wet silver–nylon control	Burn + SN + saline	10
DC control	Burn + −40 μA DC for 48 hours	10
Treatments		
−40 μA DC	Burn + −40 μA DC	110

[a] Third set of the experiment.
DC = direct current; SN = silver–nylon.

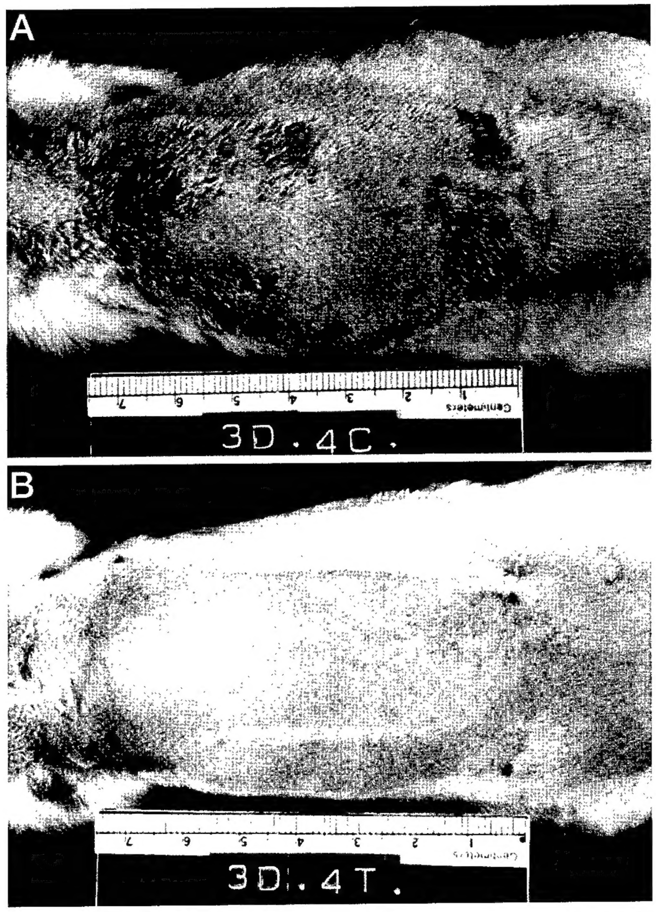

FIG 1. **Full thickness scald wound at 3 days afterburn in rats. (A) untreated control; (B) rat was treated with −40 μA direct current.**

Wound edema (E) was analyzed as excess water per gram of unburned dorsal skin (milliliters per gram).

$$E = \frac{W_b - W_n}{W_n},$$

where W_n and W_b are wet weight of normal and burned skin, respectively. Assuming that dry skin weight does not change after injury

$$E = \frac{W_b}{W_n} - 1 = \frac{P_n}{P_b} - 1,$$

where P_n and P_b are dry to wet weight ratio ($P = D/W$) for normal and burned skin, respectively.

RESULTS

Gross Examination

Gross examination of the full-thickness burn wounds showed a difference in edema formation between the untreated and DC treated animals. On day 3 postburn, edema in control wounds (Fig. 1*A*) was more obvious than in wounds treated

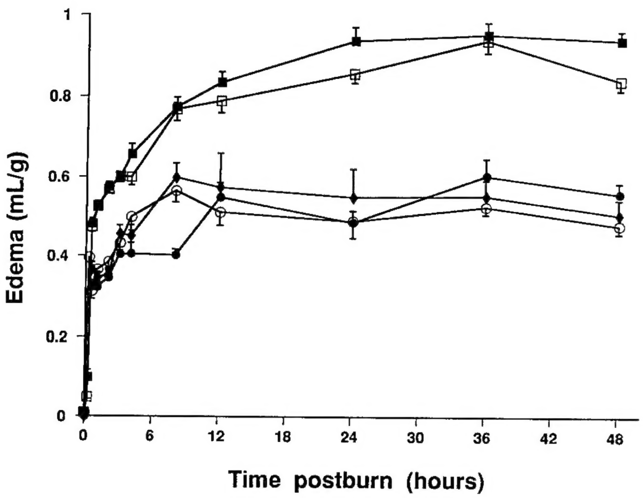

FIG 2. **Edema (mL/g of unburned skin) in full-thickness burn covering 20% total body surface area in scalded, untreated rats *(filled squares)*; rats treated with wet silver–nylon dressing *(open squares)*; rats treated with −4 μA *(diamonds)*, or −40 μA *(open circles)*, or +40 μA DC *(filled circles)*. Plotted values are mean ± SEM. $p < 0.001$ at any time after 0.5 hours postburn.**

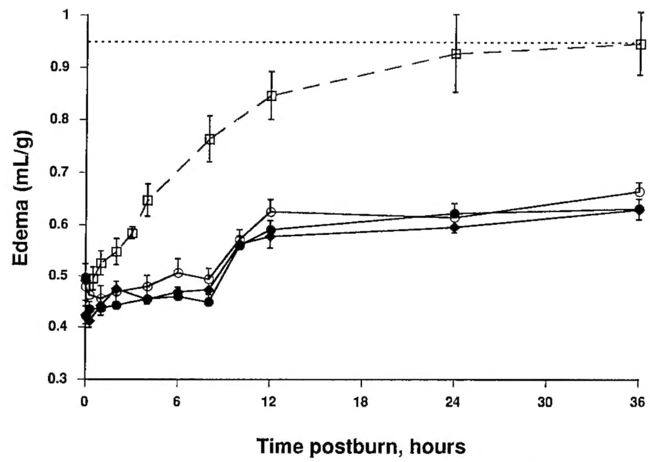

FIG 3. **Lower three curves show edema at 48 hours after burn in rats treated with −40 μA *(open circles)*, or −4 μA *(diamonds)*, or +40 μA DC *(filled circles)* applied with different lengths of delay after injury. *(Dotted line)* Edema of untreated control rats 48 hours after injury. $p < 0.001$ at any time. Upper curve shows the amount of edema in control (wet SN) burn wound *(open squares)* at each time DC application begun. Plotted values are mean ± SEM.**

with −40 μA DC (Fig. 1*B*). Small areas of desiccated eschar were seen on the surfaces of the control wounds but not on the DC treated wounds.

Edema Assay

Immediate Treatment (First Set)

Water contained in the nonburned skin of male white Sprague-Dawley rats weighing 225 ± 25 grams was 67.090% ± 0.333% (n = 30).

Figure 2 shows the kinetic curves of wound edema accumulation up to 48 hours PB in two control groups of burned rats without treatment or treated with wet SN dressing. Edema increased rapidly in the first 30 minutes (0.45 mL/g), gradually approached a maximal level (0.95 mL/g) at 36 hours PB, and then decreased. There was no significant difference in edema fluid accumulation between the two control groups ($p > 0.3$, 30 rats per each time point in each control group).

In the DC treated groups (Fig. 2), mean fluid content increased by 0.32 to 0.37 mL/g in the first half-hour PB and reached a maximal level by 8 to 12 hours PB (~0.60 mL/g). Application of DC significantly reduced wound edema at and after 0.5 hours PB by 17 to 48% ($p < 0.001$ when compared with SN control group) and this effect was similar in all DC treated groups (10 rats per time point per treatment).

After 48 hours PB, edema accumulation was obviously influenced by wound coagulation, desiccation, and inflammation. Results varied and were non-comparative among the three DC treated groups. Continuous DC application, however, was still effective at 72, 96, and 120 hours PB, causing 26 to 49% reduction of edema at the times measured ($p < 0.001$) if compared with SN control group). These data are listed in Table 4.

Delayed Application of DC (Second Set)

First series. Since continuous application of constant direct current beginning immediately after burn had pronounced effects, experiments were done to determine the effect of delaying treatment. In Fig. 3, the lower three curves show the amount of edema in the burn wound 48 hours PB when DC (−4, or −40, or +40 μA) was applied at different times PB (15 minutes to 36 hours) and maintained for the rest of the initial 48-hour PB interval. The DC reduced fluid accumulation in all of these wounds compared with SN control group (*dotted line*, $p < 0.001$). Direct current treatment started at any time within the first 8 hours was as effective as current applied immediately after injury (42 to 51% reduction of edema). When DC treatment was begun at 12 to 36 hours after injury, edema was reduced by 21 to 33%.

The upper curve in Fig. 3 shows the amount of edema fluid of control burn wounds (wet SN dressing) at each time the current application begun. At each time point, differences between the upper curve and the lower three curves equal the amount of edema fluid reduced (18 to 51%) during the period between the time of DC application and 48 hours PB.

TABLE 4. **Effect of direct current of −40, −4, or +40 μA on wound edema 3, 4, and 5 days after burn**

Time PB (hours)	BO Mean ± (SEM) (mL/g)	SN Mean ± (SEM) (mL/g)	−40 μA[a] Mean ± (SEM) (mL/g)	−4 μA[a] Mean ± (SEM) (mL/g)	+40 μA[a] Mean ± (SEM) (mL/g)
72	0.762 (0.019)	0.657 (0.023)	0.420 (0.021)	0.474 (0.019)	0.401 (0.028)
96	0.649 (0.021)	0.614 (0.023)	0.432 (0.014)	0.341 (0.029)	0.367 (0.033)
120	0.609 (0.019)	0.542 (0.020)	0.400 (0.015)	0.277 (0.021)	0.378 (0.017)

[a] $p < 0.001$ compared with burn control and wet silver–nylon control.

PB = post burn; BO = burn only control; SN = silver–nylon.

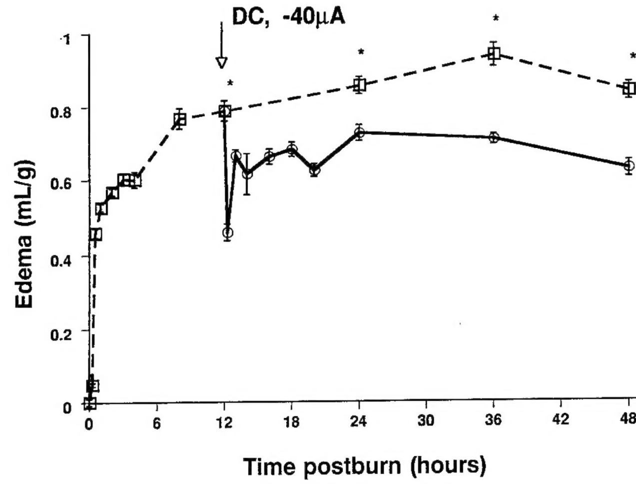

FIG 4. **Edema of 20% total body surface area full-thickness burn in rats treated with SN (*open squares*) or −40 μA DC applied after a 12-hour delay (*open circles*). Plotted values are mean ± SEM (* = $p < 0.001$).**

Second series. To measure time characteristics of the DC effect, the kinetics of edema formation were observed after application of −40 μA DC at 12 hours PB. These data are presented in Fig. 4. Current caused reabsorption of 42% of accumulated edema fluid as early as 15 minutes after application and then maintained the edema fluid level at approximately 0.66 mL/g of unburned tissue weight at all time points after the 15-minute mark.

Treatment Time Dependency (Third Set)

Experiments were done to ascertain how long DC application was required for sustained effect. A current of −40 μA DC was applied immediately after burn and discontinued at selected PB times (15 minutes to 36 hours). Water content was measured 48 hours PB. These data are presented in Fig. 5 and show that DC treatment continued for 8 to 12 hours after burn and then disconnected, produced approximately the same effect on edema as continuous treatment for 48 hours. Shorter applications produced less effect.

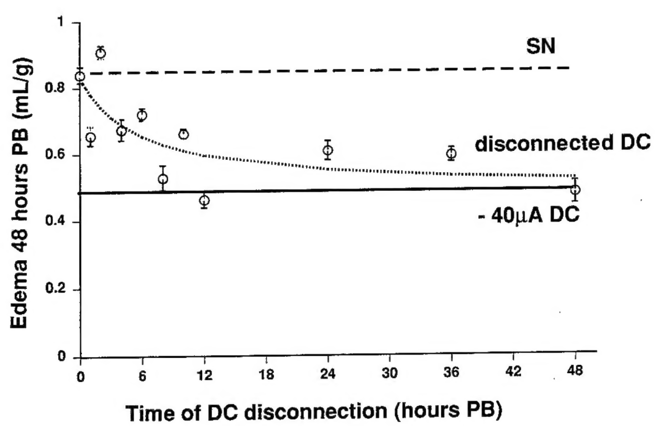

FIG 5. **Edema at 48 hours after burn in rats treated with −40 μA DC applied immediately after burn and disconnected at different times (*open circles* and *dotted line*). Plotted values are mean ± SEM. Edema level at 48 hours after injury of rats treated with −40 μA DC (*solid line*). $p < 0.001$ (disconnected DC vs. SN) at any time except 1 hour after burn.**

DISCUSSION

The phenomenology and pathophysiology of burn wound edema have been extensively studied.[14–17] The widely differing rates of water accumulation reported in burn wounds may be explained by differences in animals, burn wound models, and animal care. We used full-thickness scald burns in rats as a model. Previous experiments have shown that after 48 hours postburn coagulation, desiccation and inflammation occurring in full-thickness burns influence experimental results; therefore, observations during the first 48 hours after injury were emphasized. To avoid variation caused by environmental factors, all experimental animals were monitored at standard temperature and humidity in individual cages in the animal intensive care unit.

Analysis of fluid accumulation in the control groups showed that silver–nylon alone did not affect edema formation (Fig. 2). In both control groups (burn only and wet SN) the rate of edema accumulation was highest in the first half-hour PB and then dropped tenfold, but further accumulation of edema continued until 36 hours PB. Direct current reduced edema in such wounds by 17 to 48% at the times measured, and accumulation of edema fluid in treated wounds ceased at 8 to 12 hours PB. The differences among DC treatment groups were small. The effects of DC treatment were polarity independent, and 4 μA per total wound area (0.1 μA/cm^2) produced the same effect as 40 μA (1 μA/cm^2) current. These results are consistent with our observations of wound healing in deep second degree burns.[11]

It was necessary to continue treatment for at least 8 hours after injury or to start treatment no later than 8 hours PB to achieve maximal reduction of edema accumulation. Earlier disconnection or later application reduced the effect of the treatment, though significant reductions of edema were still observed. The effect of delayed treatment is intriguing: only two levels of DC effect were seen, a reduction of approximately 45% with 0 to 8 hours delay, and approximately 25% with delays of 12 to 36 hours. This finding suggests the possibility that two independent mechanisms involved in edema formation may be affected by DC, one early and the other later and more prolonged.[18,19] Applying current at any time between 0 and 8 hours after burn inhibits both early and late edema accumulation, while later application affects only the more prolonged phase.

On the basis of these data, we cannot determine whether the decrease of edema in the early phase (0–8 hours PB) is caused by a reduction in extravasation, enhanced reabsorption, or both (Figs. 3 and 4). To determine that, experiments with labeled plasma components would be necessary. It is clear, however, that DC application reduces edema volume when initiated between 12 and 48 hours PB, and that this effect develops within 15 minutes after treatment is started (Fig. 4). The experiment with application of DC at 12 hours PB was repeated with a smaller number of animals. The results were the same at 15 minutes after application of DC as the first series. We do not know yet how to explain this interesting finding, but it is beyond a doubt worth further study.

CONCLUSIONS

1. Immediate and continuous application of −40, −4, or +40 μA direct current through silver–nylon dressings reduced edema fluid accumulation in full-thickness burn wounds. At least 8 hours of treatment was required to achieve a sustained maximum effect.

2. The effect of direct current on burn wound edema was independent of electrode polarity and appears to be independent of current density above level of 0.1 $\mu A/cm^2$.

3. Edema was reduced if DC application was delayed as much as 36 hours afterburn. The greatest reduction of edema occurred when DC treatment was begun within 8 hours after burn, and continued through the remainder of the 48-hour study period.

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